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Drought- and ABA-Induced Changes in Polypeptide and mRNA Accumulation in Tomato Leaves[1]

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ABSTRACT

Drought stress triggers abscisic acid (ABA) biosynthesis resulting in ABA accumulation. The ABA-deficient tomato mutant, *flacca* (*Lycopersicon esculentum* Mill. cv Ailsa Craig), does not synthesize ABA in response to drought stress. This mutant has been used to distinguish polypeptides and *in vitro* translation products that are synthesized during drought stress in response to elevated ABA levels from those that are induced directly by altered water relations. A set of polypeptides and *in vitro* translation products was synthesized during drought stress in the wild type. These polypeptides and *in vitro* translation products were synthesized to a lesser extent in the drought-stressed ABA-deficient mutant. Treatment of *flacca* with ABA resulted in the synthesis of the drought-stress-induced polypeptides and *in vitro* translation products. These results support the hypothesis that many of the polypeptides that are synthesized during drought are regulated by alterations in ABA concentration. Similarly, the mRNA population was altered by ABA during drought stress.

During drought stress, leaf ABA concentration increases dramatically. This increased ABA serves to close stomata, but other roles of this large increase in ABA are unknown. The experiments reported here address the possibility that ABA induces specific genes during periods of stress. During drought and salt stress, changes in gene expression have been documented (1, 4–7, 9, 10, 17–21). Induction and repression of genes during drought stress may be a direct response to environmental conditions and/or a response to changes in hormone concentration. Alterations in ABA concentration during drought stress may serve to coordinate drought responses and the adaptation of the plant to the environment.

The tomato mutant, *flacca*, is wilty due to a lack of stomatal closure (2, 22). This is correlated with decreased ABA content (13) and can be reversed with ABA application (23). Therefore, this mutant has been used to implicate ABA's role in stomatal closure. *Flacca* has 26% of the ABA of the wild type when it is well watered (13), and leaves do not produce additional ABA upon drought stress (13). Therefore, ABA does not accumulate during drought stress in the mutant as it does in the wild type.

In this study, the pattern of polypeptide synthesis and mRNA accumulation in tomato was determined in response to drought stress. The ABA-deficient mutant, *flacca*, was used to determine if specific proteins and mRNAs accumulate in response to stress and if any of these proteins are synthesized in response to elevated ABA concentrations.

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MATERIALS AND METHODS

Plant Material. Plants of *Lycopersicon esculentum* cv Ailsa Craig and the isogenic ABA-deficient mutant *flacca* (obtained from Dr. J. W. Maxon Smith at the Glasshouse Crops Research Institute in Littlehampton, West Sussex, England), were grown in a growth chamber for 2 to 3 months, and plants were well watered.

Experimental Treatments. All treatments were completed on detached tomato leaves. Leaves were removed from the plant and immediately placed in water. Leaves were kept in water for nonstress treatments or were wilted on the laboratory bench to 88% of the original fresh weight for drought stress treatments. Leaves were maintained in a wilted state in plastic bags. Petioles were placed in 10^{-3} M ABA for ABA treatments. After a 4 h incubation period, leaflets were removed and were fed 3 μL of [^{35}S]methionine (45 μCi, 1253 Ci/mmol, Amersham) through the cut end of the petiolule. A 2 h period of incubation followed. Leaflets were then frozen in liquid nitrogen.

Protein Extraction and Analysis. Proteins were extracted from the leaf using a phenol extraction method (18). Briefly, samples were ground under liquid nitrogen and suspended in extraction buffer (0.5 M Tris-HCl [pH7.5], 0.1 M KCl, 0.05 M Na_2EDTA [pH 7.4], 2% 2-mercaptoethanol, 0.7 M sucrose). An equal volume of water-saturated phenol was added, and the samples were mixed at room temperature for 10 min. After centrifugation, the phenol phase and interface were collected. The phenol phase was reextracted with extraction buffer. Proteins were precipitated from the phenol phase with methanol containing 0.77% ammonium acetate. The pellet was washed with methanol-ammonium acetate and air dried. Incorporation of label into proteins was determined by TCA precipitation. Protein concentration was determined using the Peterson assay (15).

Samples containing 350,000 cpm were resuspended in O'Farrell (14) lysis buffer and were subjected to two-dimensional electrophoresis according to O'Farrell (14). Proteins were visualized by autoradiography after the gels were infused with PPO (0.4% PPO, 55% acetic acid, 15% ethanol, 30% xylene), washed in water, and dried.

RNA Extraction and *in Vitro* Translations. Total RNA was extracted from tomato leaves using a LiCl-phenol extraction method (16). Briefly, tomato leaves were ground under liquid nitrogen and were suspended in extraction buffer (50 mM Tris [pH 9.0], 150 mM LiCl, 5 mM EDTA, 5% SDS). An equal volume of phenol:chloroform (1:1) was added, and after centrifugation, the aqueous phase was recovered. RNA was precipitated twice in 2 M LiCl and collected by centrifugation. The isolated RNA was translated *in vitro* in the presence of [^{35}S]methionine and [^{35}S]cysteine (10 μCi Tran^{35}S-label, ICN, 1105 Ci/mmol) according to Morch *et al.* (12). *In vitro* translation products were extracted using the phenol extraction method and separated by two-dimensional gel electrophoresis (14).

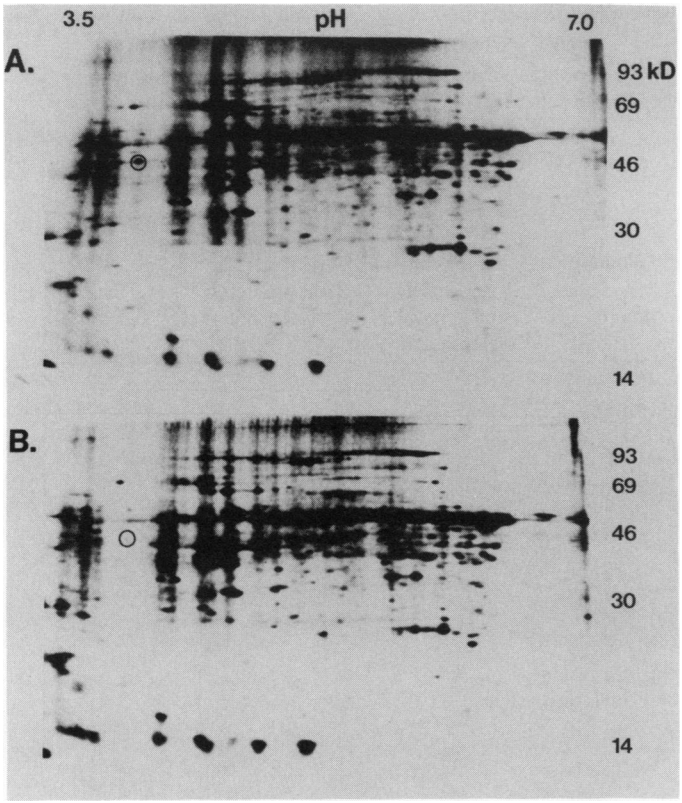

FIG. 1. *In vivo* radiolabeled polypeptides from nonstressed leaves separated by two-dimensional PAGE. Detached leaves from Ailsa Craig and *flacca* were kept in water for 4 h. Leaflets were fed [^{35}S]methionine, incubated for 2 h and polypeptides were separated by two-dimensional gel electrophoresis. Circled polypeptides were synthesized to a greater extent in Ailsa Craig. A, Nonstressed Ailsa Craig; B, nonstressed *flacca*.

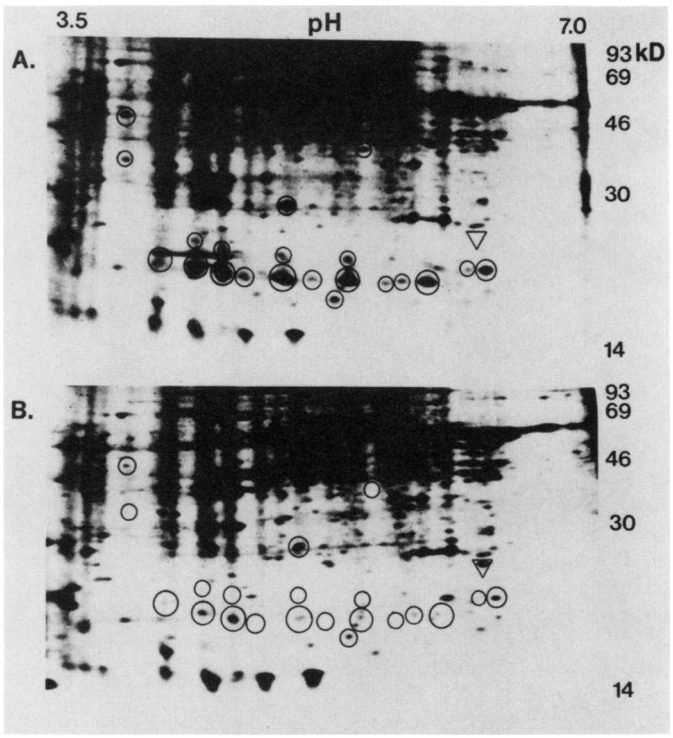

FIG. 2. *In vivo* radiolabeled polypeptides from drought-stressed leaves separated by two-dimensional PAGE. Detached leaves from Ailsa Craig and *flacca* were wilted to 88% of their original fresh weight and were then incubated in plastic bags for 4 h. Leaflets were fed [^{35}S]methionine, incubated for 2 h, and polypeptides were separated by two-dimensional gel electrophoresis. Circled polypeptides were synthesized to a greater extent in Ailsa Craig. The polypeptide marked with a triangle was synthesized to a greater extent in *flacca*. A, Drought-stressed Ailsa Craig; B, drought-stressed *flacca*.

ABA Quantitation. ABA was quantified using a competitive ABA-radioimmunoassay as described by Bray and Beachy (3).

RESULTS

***In Vivo* Labeled Polypeptides.** ABA-deficient mutant and wild-type leaflets were labeled with [^{35}S]methionine after a 4-h treatment period, and the polypeptides were separated by two-dimensional gel electrophoresis. There were no significant differences in patterns of polypeptide accumulation between the nonstressed *flacca* and wild type (Fig. 1). One polypeptide was synthesized in the wild type that was not synthesized in the mutant.

During drought stress, a set of approximately 21 radiolabeled polypeptides was synthesized in stressed wild type leaves that was not synthesized or synthesized to a lesser extent in nonstressed leaves (Figs. 1A and 2A). The majority of these polypeptides ranged from a mol wt of 18 to 24 kD with pIs from 4.25 to 6.7. There were also approximately four polypeptides of higher mol wt that were synthesized in response to drought stress. A comparison of polypeptide synthesis during drought stress was made between the wild type and the ABA-deficient mutant, *flacca*. A set of polypeptides was synthesized in the drought-stressed wild type that was synthesized to a lesser extent in the drought-stressed *flacca* (Fig. 2). Several of these polypeptides were not detected among the polypeptides of the stressed mutant. The set of polypeptides that accumulated only in the wild type was the set of drought-stress-induced polypeptides. ABA application to the wild type and the mutant resulted in synthesis of this same set of polypeptides (Fig. 3). These results suggest that a set of drought-stress polypeptides was synthesized in response to elevated ABA levels.

Total Protein Synthesis. Total protein synthesis was unaffected by drought stress and slightly reduced by ABA treatments in the wild type (Table I). Total protein synthesis was reduced by ABA and stress treatments in the mutant.

ABA Concentration. ABA accumulation was determined after drought-stress treatments using a radioimmunoassay (3). ABA synthesis was induced by drought stress in the wild type (Table II). There was a 22-fold accumulation of ABA in the drought-stressed wild type. There was a 3-fold accumulation of ABA in drought-stressed mutants. The ABA level of the mutant was approximately 50% of the wild type with nonstressed conditions and 6% of the wild type during drought stress. Feeding ABA to the leaves resulted in a large increase in ABA concentration of the leaflet to at least 60,000 ng/g fresh weight.

***In Vitro* Translation Products.** RNA isolated from nonstressed wild type and mutant leaves was translated *in vitro* in wheat germ extract in the presence of [^{35}S]methionine and [^{35}S]cysteine and separated by two-dimensional gel electrophoresis. One *in vitro* translation product was synthesized from RNA isolated from the nonstressed mutant that was not synthesized from RNA isolated from the wild type (Fig. 4). The pattern of *in vitro* translation products was compared between nonstressed and stressed wild type. A set of *in vitro* translation products was synthesized from RNA isolated from stressed wild type leaves that was not synthesized or synthesized to a lesser extent from RNA isolated from the nonstressed leaves (Figs. 4A and 5A). Comparisons between the genotypes showed that there were also *in vitro* translation products that were synthesized from RNA

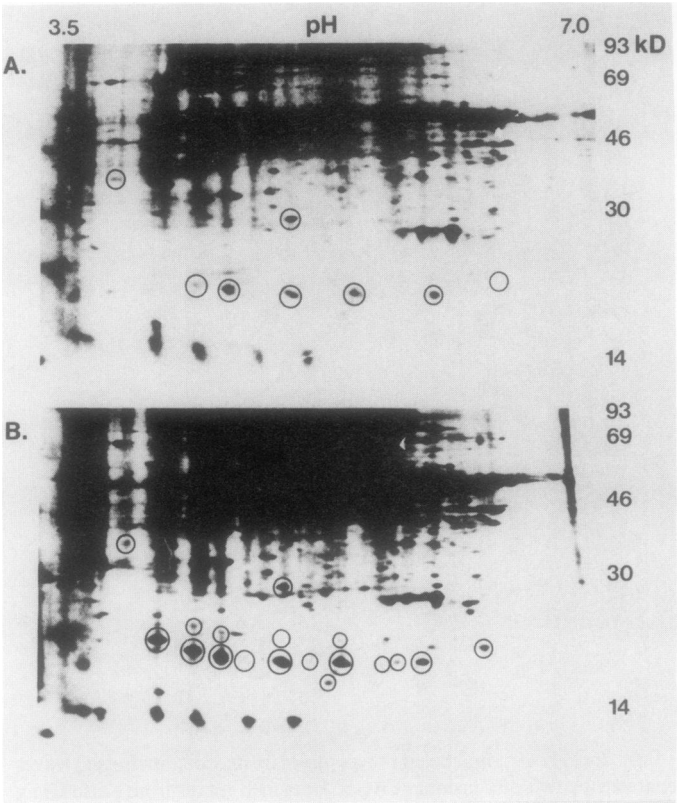

FIG. 3. *In vivo* radiolabeled polypeptides from ABA-treated leaves separated by two-dimensional PAGE. Detached leaves were treated with 10^{-3} M ABA for 4 h. Leaflets were fed [^{35}S]methionine for 2 h and polypeptides were separated by two-dimensional gel electrophoresis. Circled polypeptides were not synthesized in nonstressed leaves and correspond to the drought-induced polypeptides. A, ABA-treated Ailsa Craig; B, ABA-treated *flacca*.

Table I. *Effect of Drought Stress and ABA Treatments on Protein Synthesis of Detached Tomato Leaves of the Wild Type (Ailsa Craig) and the ABA-Deficient Mutant (flacca)*

[^{35}S]Methionine was fed to the leaflets of both genotypes after 4 h of the different treatments. Incorporation into protein was determined by TCA precipitation after a 2-h incubation period. Average and SE of six replications.

Genotype	Treatment	Percent of Nonstressed Control
		% ± SE
Wild type	Nonstress	100
	ABA	93 ± 3
	Water deficit	97 ± 14
flacca	Nonstress	94 ± 8
	ABA	85 ± 10
	Water deficit	68 ± 11

isolated from drought-stressed wild type that were synthesized to a much lesser extent from the RNA isolated from the stressed mutant (Fig. 5). The *in vitro* translation products that were synthesized only in the wild type were the same products that were newly synthesized during periods of stress. The treatment of leaves with ABA for 4 h prior to RNA isolation resulted in the accumulation of this same set of *in vitro* translation products in the mutant and wild type that was produced in the wild type during drought stress (Fig. 6).

Table II. *ABA Concentrations of Detached Tomato Leaflets from Wild Type (Ailsa Craig) or ABA-Deficient Mutant (flacca)*

ABA was quantified using an ABA-radioimmunoassay. Average and SE of four experiments.

Genotype	Treatment	ABA	Percent of Wild-type Nonstress
		ng/g fresh wt ± SE	
Wild type	Nonstress	108.4 ± 12.7	100
	Water deficit	2308.5 ± 159.0	2130
flacca	Nonstress	57.0 ± 8.4	54
	Water deficit	145.8 ± 18.5	150

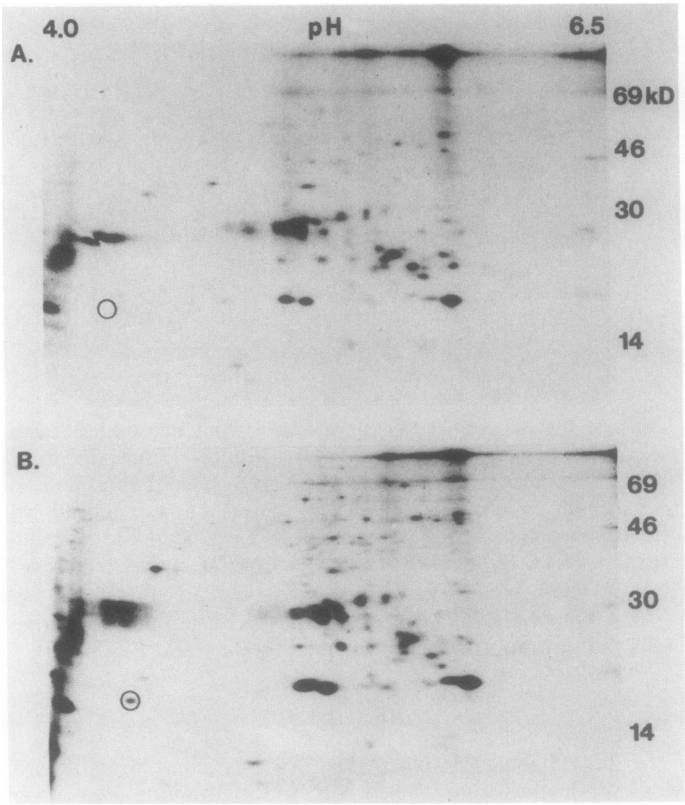

FIG. 4. *In vitro* translation products of mRNA from nonstressed leaves separated by two-dimensional PAGE. Detached leaves were incubated for 4 h in water, RNA was isolated from the leaves and was *in vitro* translated in the presence of [^{35}S]methionine and [^{35}S]cysteine. *In vitro* translation products were separated by two-dimensional gel electrophoresis. The circled *in vitro* translation product was synthesized to a greater extent in *flacca*. A, Nonstressed Ailsa Craig; B, nonstressed *flacca*.

DISCUSSION

Plants respond to alterations in the environment through changes in metabolic pathways and alterations in other physiological and developmental responses. One means of response is an alteration of synthesis of specific polypeptides. New polypeptides are synthesized in response to drought, salt, and temperature stress (9, 11, 17, 18). This report also identifies a set of polypeptides that are synthesized in response to drought stress in tomato. It is unknown if these polypeptides are involved in adaptation to the stress, and the mechanism of induction is not understood.

During drought stress, the ABA concentration of the plant increases significantly. Neill and Horgan (13) reported that *flacca* does not produce additional ABA when it is stressed. However, there was a small increase in ABA concentration of *flacca* during

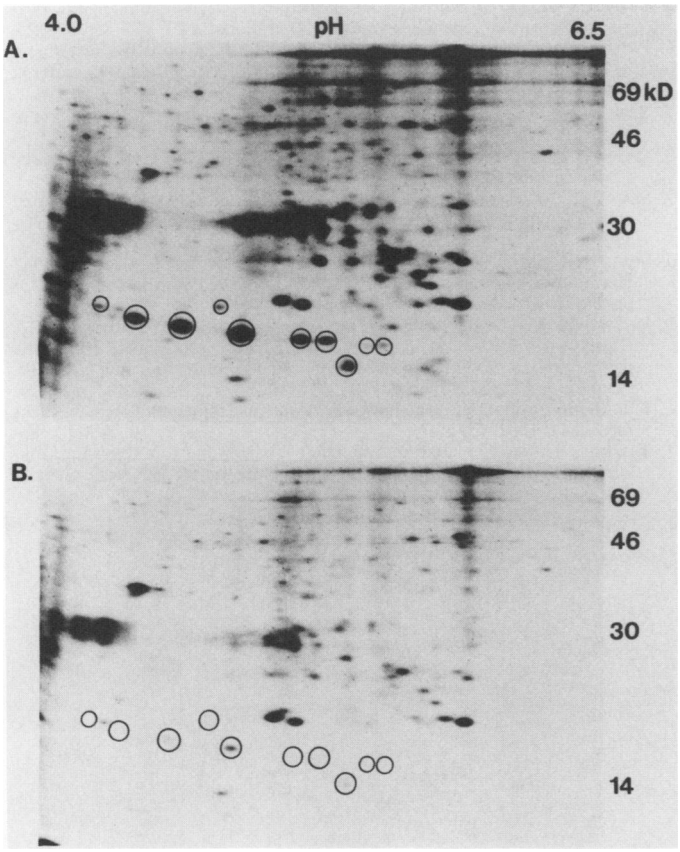

FIG. 5. *In vitro* translation products of mRNA from drought-stressed leaves separated by two-dimensional PAGE. Detached leaves were drought stressed for 4 h, RNA was isolated from the leaves and *in vitro* translated in the presence of [^{35}S]methionine and [^{35}S]cysteine. *In vitro* translation products were separated by two-dimensional gel electrophoresis. Circled *in vitro* translation products were synthesized to a greater extent in Ailsa Craig. A, Drought-stressed Ailsa Craig; B, drought-stressed *flacca*.

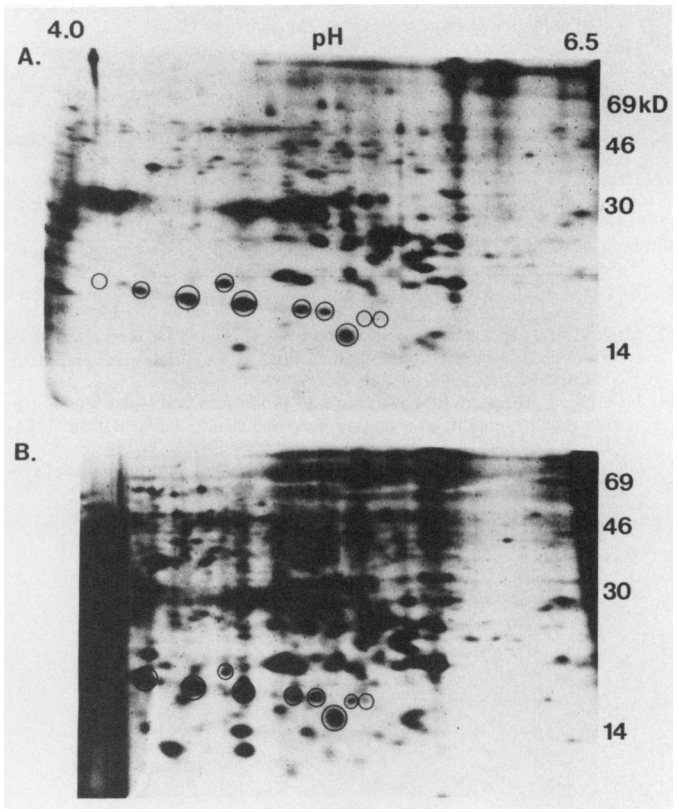

FIG. 6. *In vitro* translation products of mRNA from ABA-treated leaves separated by two-dimensional PAGE. Detached leaves were treated with 10^{-3} M ABA for 4 h, RNA was isolated from the leaves and *in vitro* translated in the presence of [^{35}S]methionine and [^{35}S]cysteine. *In vitro* translation products were separated by two-dimensional gel electrophoresis. Circled *in vitro* translation products correspond to the drought-induced *in vitro* translation products. A, ABA-treated Ailsa Craig; B, ABA-treated *flacca*.

drought stress in these experiments. The difference in these results may be due to the environmental conditions in which the plants are grown or the experimental protocols.

An increase in ABA during drought stress is thought to cause stomatal closure, but it is unknown if there are any additional responses to the increased ABA concentration. The use of an isogenic mutant has made it possible to show that the changes in polypeptide synthesis during stress are probably a result of increased ABA levels. The ABA-deficient mutant *flacca* produced a decreased amount of ABA when it was stressed and synthesized a specific set of drought-induced polypeptides to a much lesser extent. Because *flacca* did produce some ABA during stress, the limited production of stress proteins was expected. It is suggested that elevated ABA levels during drought stress result in induction of a specific set of ABA-induced polypeptides. The nature and role of the polypeptides during stress is currently unknown.

It is generally thought that total protein synthesis is decreased by stress (8). However, total protein synthesis was only slightly reduced by drought stress in these experiments and the proteins that were synthesized during periods of nonstress were not significantly reduced.

The accumulation of proteins in response to ABA may be regulated from transcription to accumulation of the mature gene product. *In vivo* labeling studies have shown that there is a difference in accumulation of gene products during stress in the mutant compared with the wild type. However, these studies do not address the level of the regulation. *In vitro* translation products were used to show that there were differences in accumulation of mRNAs during drought stress among the mutant and the wild type. A set of mRNAs was increased during drought stress in response to an increase in the ABA concentration. This accumulation of specific mRNAs may be due to increased transcription or altered post-transcriptional processing that promote specific mRNA accumulation. However, this does not eliminate the possibility that ABA regulates gene expression at the translational level; it merely suggests that some regulation occurs at the level of mRNA accumulation. In order to continue to study the mechanism of gene regulation during drought stress by ABA, genes that are induced by ABA are currently being isolated.

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